REVIEW

The Complexity of the Epstein-Barr Virus Infection in Humans

Eva KLEIN

Microbiology and Tumor Biology Center (MTC), Karolinska Institutet, Stockholm, Sweden

The Epstein-Barr virus (EBV) was isolated 40 years ago from cultures of Burkitt lymphoma cells (BL). The tumor was encountered in Africa and exhibited characteristical geographical, clinical and pathological features. Serological studies revealed that the virus is ubiquitous in humans. The primary infection is often accompanied by the syndrome of acute infectious mononucleosis (IM). It can induce malignant proliferation of B lymphocytes in conditions of immunodeficiency. EBV can immortalize B lymphocytes in culture. These cells carry the virus as episomes and express 9 virally encoded proteins. Their immunological recognition constitutes the surveillance which is responsible for the healthy virus carrier state. The main virus reservoir is represented by a low number of resting B lymphocyte which contain the viral genome but do not express its transformation proteins. The viral genom is detectable in all African BLs, in variable proportions of nasopharyngeal carcinoma, Hodgkin's disease, T cell lymphoma, lymphoepithelial like carcinoma, gastric carcinoma and leiomyosarcoma cases. The role of EBV in the genesis of these tumors is unknown. (Pathology Oncology Research Vol 4, No 1, 3–7, 1998)

Key words: EBV, infections mononucleosis, immune response

History of the discovery of EBV

A hitherto unknown childhood lymphoma most frequently localized to the jaw was described in 1958 by the surgeon Dennis Burkitt when he worked in Uganda.[4] The patients came from an area of Africa where holoendemic malaria was common. D.B. suspected that an insect-transmitted agent contributes to the pathogenesis of this tumor which received the name: Burkitt lymphoma, BL.

Herpes like particles were detected by electron microscopy in *in vitro* cultures of the tumor tissue (but not in the *ex vivo* tumor cells). The cells' surfaces carried immunoglobulin molecules and this feature identified them to be B lymphocytes. The BL led thus to the discovery of the first herpes virus with connection to malignancy. After the discoverers it was named Epstein-Barr virus, EBV. Burkitt lymphoma patients had high titers of antibodies directed against the viral capsid. The viral genome and virus specific proteins localized in the nucleus – EBNA, EBV nuclear antigen – were detected in the tumor cells. Virus collected from supernatants of the BL cell cultures transformed human B lymphocytes *in vitro* and from such cultures immortalized lymphoblastoid lines; LCLs could be established which contained episomal EBV genomes. The hypothesis was proposed therefore that EBV is directly responsible for the genesis of BL. However this assumption was abandoned rather soon when clinically and histopathologically similar, but EBV negative tumors were encountered outside the African endemic area.

Both the EBV positive and negative tumors carried a characteristical chromosomal translocation, involving either chromosome 14, 2 or 22 and chromosome 8 which juxtaposes the immunoglobulin (most often heavy chain, chr. 14) and the myc (chr 8) genes. This constellation leads to the immunoglobulin gene directed constitutive expression of the myc gene which drives the cells to proliferate. The discovery seemed to clarify the mech-

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Correspondence: Eva KLEIN, Microbiology and Tumor Biology Center (MTC), Karolinska Institutet, Stockholm, Sweden, S-171 77.

Abbreviations: BL, Burkitt lymphoma; LCL, lymphoblastoid cell line; EBV, Epstein-Barr virus; EBNA, Epstein Barr virus encoded nuclear antigen; LMP, latent-membrane-protein; NPC, nasopharyngeal carcinoma; IM, infectious mononucleosis; CTL, cytotoxic T lymphocytes; MHC, major histocompatibility comples; PCR, polymerase-chain-reaction; PTLD, post-transplant lymphoproliferative diseases

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anism of tumor development but at the same time the role of EBV in the genesis of BL became – and it is still an enigma.[18]

Infectious mononucleosis

Serological studies have shown that EBV is ubiquitous in humans. The antibody studies led to a surprising turn. A serendipitidous observation followed by planned strategical studies incriminated EBV to be the cause of acute infectious mononucleosis (IM).[12] The disease occurs at primary infection in about half of the individuals, but not in children whose infection is usually subclinical. Virus particles in the saliva, present most abundantly during the acute infection, but also intermittantly in the virus carrier healthy individuals are the main source of transmission, occurring most often in adolescents.

A typical symptom of IM is lymphocytosis made up by activated T cells. Most likely, this is the manifestation of the T cell response against virus infected activated B lymphocytes. The lymphoid tissue of the patients contains cells in which viral DNA and its products can be detected. These cells are: a few small B lymphocytes, many B blasts and a few giant cells which are similar to Reed-Sternberg/Hodgkin's cells. After the disease dissolves the virus can be detected in the tissues and in the blood only with the highly sensitive PCR method. Irrespective whether the primary infection is accompanied by IM or it occurs without symptoms, the individuals become virus carriers for lifetime with manifestation of EBV specific humoral and cellular immunological memory.

Virus in the tissues can be detected also by its capacity to immortalize B lymphocytes *in vitro*. When blood lymphocytes or lymphocyte containing tissues from seropositive individuals are explanted LCL-s can arise after a few weeks of culturing. However the transformation of the culture occurs only if the function of T lymphocytes is abrogated either by their elimination or by inhibitiory measures.[20]

Immunological control of the EBV infection

The above mentioned *in vitro* experiments and the accumulated clinical experience established that an unimpaired immunological function is essential for the benign nature of the primary infection and for the ensuing usually undisturbing EBV carrier state.

The current notion is that among the various parameters of immunity, the MHC Class I restricted cytotoxic T cells (CTL) are the most important players. The CTLs generated *in vitro* recognize peptides derived from several virally encoded proteins which can be expressed in the B lymphocytes.[23]

The main virus reservoir in healthy individuals seems to be a low number of cells in the resting B lymphocyte population.[5] Their existence in the face of the EBV specific immunity is allowed by *1.* the passivity of the resting B cell when it interacts with T lymphocytes and *2.* the strategy of the virus with regard to the expression of its genes.[15,16,17]

- *re 1.* Resting B lymphocytes do not interact efficiently with T cells. It seems that they lack several features which are required for this function. When activated the expression of MHC, intercellular adhesion and costimulatory surface molecules increases and they produce immunoregulatory cytokines.
- *re 2.* Expression of the transformation associated EBV encoded proteins in the B cells with latent infection is regulated by the phenotype, whether they correspond to the resting or activated state. Most importantly, the expression of the nuclear proteins – EBNAs – is controlled by different promoters in these two conditions. Only one of these, EBNA-1, is expressed in the B cell with the resting phenotype. EBNA-1 does not drive the cells for proliferation. The EBV positive BL cells look like resting B cells and they express only EBNA-1. In culture however their phenotype changes they acquire activation markers and further EBV encoded proteins are detectable.

The restricted expression of the EBV genome in the resting B cells is probably the key for the „harmless" virus presence in health.[15,16,19] Genetic analysis of the virus with regard to its capacity to transform normal B cells has determined that the expression of 6 EBV encoded proteins are essential for induction of proliferation.[7] One of these, LMP-1, is localized in the plasma membrane and 5 proteins are nuclear; EBNA 1,-2,-3,-5 and 6. Three further proteins, LMP-2a, LMP-2b and EBNA-4 and two small RNA transcripts (EBERs) do not play a role in the *in vitro* immortalization event. When all these proteins are expressed, the virus-cell interaction is referred to as Type III which is the characteristics of the *in vitro* immortalized B cell, LCL, and of the EBV positive BLs carried in culture. Such cells express markers of B cell activation. The expression of transformation related proteins and the activation markers accompany each other. Such cells are readily recognized by T lymphocytes. In mixed cultures LCLs strongly stimulate T cells. The strong reaction of T cells against LCL (even autologous) occurs even in experiments with cells of seronegative individuals in which recognition of EBV encoded antigens do not occur. In experiments with cells of seropositive individuals the EBV specific cellular memory is manifested by the appearance of specific CTLs which recognize several virally encoded proteins in a MHC Class I restricted manner.[19,23]

The functions of the latency associated viral proteins EBNA 1,2,3,5,6 and LMP-1 are largely but not completely known.[6,7,24] Expression of the plasma membrane localized LMP-1 imposes several changes on the B cells, while it is pivotal for the immortalization it requires the presence of the other EBV proteins. LMP-1 induces expression of cellular adhesion molecules (important for interaction with T cells) and it induces bcl-2 (important for protection from apoptosis).[11,25] The function of the LMP-1 molecule is similar to the ligand activated CD40 molecules and to the tumor necrosis factor receptors.[9]

The virus carrier state

The viral genome positive resting small B lymphocytes in the healthy individuals express only EBNA-1.[5] This protein does not disturb the fate of the virus genome carrier normal B cells in the face of EBV specific immune response. EBNA-1 is expressed in all cells which carry the viral genome. The resting EBNA-1 positive B cell does not interact with T lymphocytes. The protein does not impose antigens which can be recognized by MHC Class I restricted cytotoxic T cells. This is due to the presence of a glycin alanine repeat in the molecule which inhibits its processing.[17] Consequently peptides derived from EBNA-1, which associated with the Class I molecules serve as targets for CTL do not appear on the cell surfaces. When the B cells are activated additional EBV encoded proteins may be expressed and these, together with the change to the blast phenotype impose sensitivity to the cell mediated immune response.

The pathways which operate in the presentation of peptides by the MHC Class I and Class II molecules differ. The helper T cell function and the Class II dependent presentation of the EBNA-1 molecule functions properly and EBNA-1 reactive antibodies are produced regularly in the virus carrier individuals.

The following scenario can be envisaged: very few EBV genome positive normal B cells exist in healthy individuals. These cells do not attract the attention of the EBV specific cellular memory as long as they are not transformed to blasts. If this occurs e.g. when they participate in responses to antigen, they may express the transforming proteins and may therefore represent a danger. However since these proteins provide antigenic peptides, they can be targets for EBV specific cellular immunity and therefore the cells are eliminated in healthy individuals.[15,19,24]

The dangers of the EBV carrier state

The elevated risk for EBV positive B cell malignancies in individuals whose immune system is impaired provides evidence for the validity of this picture.

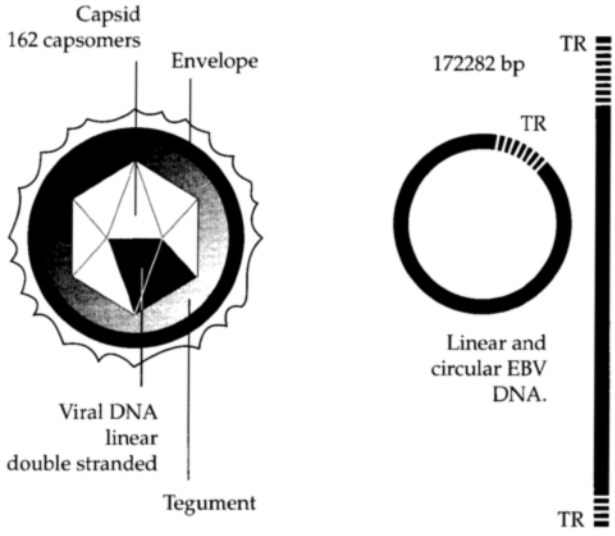

Figure 1. EBV is a large, enveloped herpesvirus encoding over 80 proteins. The envelope is a cell derived lipid bilayer into which viral glycoprotein (gp350/220) spikes are inserted. In the virus particle the genome is linear with several terminal repeates (TR) on both ends. After infection of B cells the genome becomes episomal. Circularisation is brought about by the interaction of the two TRs. Importantly the joining TRs provide various TR numbers which will be maintained in the progeny of the cell. The size of the TR fragment is thus diagnostic for the history of a virus genome carrying population. In this way its clonality and identical origin can be detected (From KI Falk doctoral dissertation; Karolinska Institutet, Stockholm, 1997.)

When T cell immunity is severly compromized, activated EBV carrier B lymphocytes may expand because of the lack of the immunological control. This results in polyclonal and ultimately monoclonal, life endangering lymphoproliferations. These EBV associated virtually malignant disorders are often lethal complications of genetically determined pronounced deficiencies in cellular immunity, in AIDS and they risk the life of immunosuppressed recipients of bone marrow and organ allografts.[8] The histopathology of post-transplant lymphoproliferative diseases, PTLD, varies with a spectrum starting with reactive or hyperplastic looking morphology to that of immunoblastic lymphoma. The majority of the lymphomas are EBV positive.

The incidence of PTLD varies according to the intensity of the immunosuppressive regimen. Bone marrow transplant patients represent a special category. The lymphomas develop soon after transplantation and the risk is confined to a relatively short interval (1–7 months) which corresponds to weakness in T cell reactivity. After this time a functioning T cell population is recovered from the small number of T cells in the graft and the risk subsides. The tumors in the bone marrow recipients are almost invariably of donor cell origin. Estimation of the

number of EBV positive B cells in the transplants indicated that these expand with striking pace in the new host. The knowledge about the EBV specific cellular response and the possibility to generate specific CTLs *in vitro* motivated therapeutical strategies using adoptive transfer of T cells from the graft donor and these were often beneficial.[21]

EBV in non-B cell malignancies

During several years after its discovery EBV was thought to be associated exclusively with B lymphomas and with the epithelial cells of nasopharyngeal carcinoma (NPC). In the latter the cells were shown to harbor the viral genome and to express only EBNA-1 among the nuclear proteins. About half of the tumor cases expressed also LMP-1. EBNA-1 and LMP-1 expression is designated as Type II virus cell interaction. In spite of the long time during which intensive effort was invested to solve the problem, the mechanism by which the virus contributes to the genesis of NPC is still not clarified. Two obstactles contribute to the elusiveness of the problem: 1. epithelial cells cannot be infected *in vitro* with EBV and the EBV receptor, CD21, a marker of B lymphocytes, is not expressed on epithelial cells; 2. cell lines derived from NPC which harbor EBV do not exist and the NPC tumors which grew out from the grafts in nude mice have lost the viral genome.

With the development of highly sensitive techniques, including immunostaining of solid tissues, the list of tumor types among which EBV positive ones are encountered increased. Now it includes a considerable proportion of Hodgkin's disease, a fraction of T cell lymphomas (interestingly, the frequency depends on their localisation), a fraction of lymphoepithelial like carcinomas, gastric adenocarcinomas and the rare leiomyosarcomas which occur in AIDS patients.[1,2,3,13,14,22,24] The pattern of the expression of EBV genes in these tumors is similar to NPC in that they express only EBNA-1 among the nuclear proteins and they may or may not express LMP-1.

In recent experiments using an EBV strain into which a selective marker was introduced, the infection of cell lines derived from human gastric carcinomas was achieved. The virus was shown to use a receptor which differs from the CD21 of B lymphocytes. Similarly to tissue specimens of gastric carcinomas, the *in vitro* infected cell lines expressed EBNA-1, and some expressed also LMP-1.[26] Hopefully this system will help to understand at least some details about the interaction of EBV and epithelial cells.

The exceptional behaviour of the EBNA-1 molecule among the EBV encoded proteins in that it does not elicit a CD8 CTL response may explain why the non-B cell tumors can arise in immunocompetent individuals. However in experiments with B cells LMP-1 can elicit specific CTL responses and could be expected therefore to be the target for a surveillance mechanism. The immune response against the EBV genome carrier tumors which originate from other than B lymphocytes requires further investigation.

These new findings change the view concerning the nature of the EBV-human relationship. The role of EBV in the genesis of the non-B cell tumors is unknown. It is possible that the general virus carrier state in humans cannot regarded anymore as a largely "harmless" parasitism.

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